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# AGE OF PUBERTY AND FERTILITY OF MALE NEW ZEALAND WHITE RABBITS ORALLY ADMINISTERED WITH ROYAL JELLY OR/ AND BEE HONEY

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## ABSTRACT

The present study was designed to investigate the effects of royal jelly (RJ) or/ and bee honey (H) on age of sexual puberty, semen quality and fertility of male New Zealand White (NZW) rabbits. Eighty pre-pubertal male NZW rabbits and 80 hybrid nonparous female rabbits were used in the present study. Male rabbits were randomly divided into 4 groups (20 bucks per group), bucks were administered orally with 0.5 mL of a solution/ kg body weight (BW), 3 times weekly for 6 weeks; which contained: 1) water for control (1st group), 2) 0.25 mL bee honey + 0.25 mL water (2nd group), 3) 200 mg royal jelly + 0.5 mL water (3rd group) and 200 mg royal jelly + 0.25 mL honey + 0.25 mL water (4th group).

The results of the present study showed that pre-pubertal NZW rabbits received RJ or/ H and showed significantly earlier ($P<0.05$) puberty age (earlier ages at testis descending into scrotum, separation of penis from sheath, beginning of fighting, first ejaculated sperm, appearance of sperm in testis and epididymis); decrease ($P<0.05$) of reaction time, increase of ejaculate volume, percentage of sperm progressive motility, sperm-cell concentration and seminal plasma fructose concentration compared to control group. On the other hand, percentages of dead and abnormal spermatozoa increased significantly ($P<0.05$) for control group compared to the other three groups. Blood plasma concentrations of testosterone and cholesterol increased significantly ($P<0.05$) for royal jelly or/ and bee honey groups compared with control. Otherwise, plasma activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) differed non-significantly among groups. Rabbit bucks treated with royal jelly or/ and bee honey showed better fertility (higher conception rate and litter size) than control bucks.

In conclusion, the results of the present study showed that oral administration of royal jelly or/ and bee honey at any level could be used beneficially to have earlier puberty age, improve semen quality and fertility of male NZW rabbits. This improvement was also mirrored on better liver functions as observed with normal concentrations of AST and ALT.

**Keywords:** Rabbit bucks, fertility, bee honey, puberty, rabbits, royal jelly, puberty semen.

## INTRODUCTION

Rabbit is an excellent laboratory animal which offers many advantages in the field of reproduction. It is easy to handle owing to its size (Naughton *et al.*, 2003). Developing and maintaining maximum reproductive efficiency in rabbits is important to the economy of the rabbit industry. Age at sexual puberty is one of the most important parameters affecting the reproductive efficiency of farm animals. Asdell (1946) has stated that early puberty and high initial fecundity may be good bases for economic selection.

The age of puberty is influenced by environmental temperature, photo period, age and breed of animal, heterosis, body weight as affected by

nutrition, and growth rates before and after weaning (Hafez and Hafez, 2000). Different managerial treatments were established to have earlier puberty age. El-Sherbiny (1994) showed that treatment of pre-pubertal male NZW rabbits with GnRH analogue resulted in earlier age of puberty and improved their semen quality and fertility.

At last years, there are international interests concerning application of natural sources in animal production field (Hashim *et al.*, 2013).

The inclusion of bee honey and royal jelly to male animals showed semen quality improvement. Gelam honey has the potential to increase the fertility of male rats by increasing sperm count and number of spermatozoa with normal morphology (Syazana *et al.*, 2011). Honey contains high level of metabolizable energy in form of glucose and fructose (Molan and Russell, 1988; Al-Waili, 2004) ; minerals such as magnesium, potassium, calcium, sodium chloride, sulphur, iron and phosphorus; as well as vitamins B1, B2, C, B6, B5 and B3 (Estevinho *et al.*, 2008). Bee honey has health benefits due to its antibacterial, antioxidant and wound healing properties (Aljady *et al.*, 2000).

Royal jelly (RJ) is a secretion product of the cephalic glands of nurse bees that has been used for centuries for its extraordinary properties and health effects (Pavel *et al.*, 2011; Mărghitas, 2008). Many researchers studied the effect of royal jelly on male fertility. On rabbits, Elnagar (2010) concluded that RJ administration to heat stressed male rabbit bucks can counteract their "summer infertility" and improve their physiological status and also for growing rabbits (Elnagar *et al.*, 2010). Hassan (2009) found that, royal jelly is a beneficial treatment of male rats especially on sperm count and livability.

Further experimentation (*in vitro*, in animal's research) and validation would be needed to prove any useful benefit and action mechanisms of native bee honey and royal jelly and isolated compounds as well.

Therefore, this study was designed to examine the effects of royal jelly or/ and bee honey on age of puberty, semen characteristics and fertility of male NZW rabbits.

## MATERIALS AND METHODS

This study was performed from November 2013 to May 2014, at the Intensive Rabbit Production Unit, Faculty of Agriculture, Ain Shams University, Cairo, Egypt and a Private Rabbit Farm, Kalioubia, Egypt.

**Animals and experimental design:**

A total number of eighty male New Zealand White (NZW) rabbits, two months old, with average body weight of ١.25 ± 0.01 kg was used in the present study. For testing the fertility of male rabbits, 80 hybrid nonparous females aged 140 – 155 days old, with average body weight 2.87± 0.21 kg at a private rabbitry, Kalioubia governorate, Egypt, were used (20 females per each experimental group). Sexing of pre-pubertal males was done one week before treatment (53 days old). Males were kept in groups of 2-3 males together until fighting behavior; then separated individually in galvanized wire cages in a naturally ventilated

building and fed a commercial concentrate pelleted diet according to their reproductive condition recommended by NRC (1977); clean fresh water was available all the time.

Pre-pubertal male NZW rabbits were randomly divided into 4 groups (20 males per group), bucks were administered orally with 0.5 mL of a solution/ kg body weight (BW), three times weekly for 6 weeks; which contained: 1) water for control (1st group), 2) 0.25 mL bee honey + 0.25 mL water (2nd group), 3) 200 mg royal jelly + 0.5 mL water (3rd group), and 200 mg royal jelly + 0.25 mL honey + 0.25 mL water (4th group).

**Techniques and Procedure:**

**Live body weight:**

All the experimental animals were weighed weekly from the age of 60 days up to 179 days.

**Sexual behavior:**

For experimental male rabbits, age at descending of testes into scrotum, age at the beginning of fighting, age at separation of penis from sheath, and age at the first ejaculated sperm were recorded as described by Berger *et al.* (1982a and b) and El-Sherbiny (1994).

**Anatomical measurements and histological parameters:**

At ages of 60, 90, 120 and 150 days old, 3 males from each group were sacrificed for anatomical and histological measurements. Immediately after rabbits sacrificing, dissection of male genitalia was performed. Weight of the two testes and epididymis were recorded. Testicular index (length × depth × width) was calculated in $cm^3$. Values obtained were calculated as mean of left and right testis and epididymis measurements. After dissection of male genitalia, the left testis and the left epididymis from each sacrificed animal were embedded in formalin (10% v/v), then replaced after 24 h by 70% ethanol till time of making paraffin blocks and staining with eosin / haematoxilin mixture as described by McManus and Mowry (1960). Age at appearance of spermatozoa in seminiferous tubules of testis and in epididymal ducts was recorded.

**Blood collection and plasma Biochemical analysis:**

Blood samples were collected from the marginal ear vein of male rabbits using heparinized tubes on ages of 6o, 75, 90, 105, 120, 135 and 150 days old. Blood plasma were separated by centrifugation at 700 × g for 20 min and stored at -20°C until analysis as described by Hashim *et al.* (2013).

Blood plasma activities of aspartate amino transferase (AST), alanine amino transferase (ALT) and cholesterol were determined using colorimetric method by commercial kits obtained from Biodiagnostic, Dokki, Giza, Egypt. Plasma testosterone concentration was measured using solid-phase enzyme immuonoassay (Elisa) kits for rabbits obtained from Chemux Bioscience, INC., San Francisco, USA. The lower limit of assay detection was 0.1 ng / mL and the upper limit was 18.0 ng / mL. The intra-and inter- assay coefficients of variation (CV) were 9.6 and 6.1 %, respectively.

**Semen collection and evaluation:**

Semen was collected artificially from each buck once weekly. Two successive ejaculates were collected; each ejaculate was kept separated for examination. The average values of both first and second ejaculates' were calculated. The parameters examined semen quality of rabbit males were:

reaction time (seconds); pH values using pH comparative papers; Ejaculate volume (mL); percentage of sperm progressive motility; percentage of dead spermatozoa assessed by nigrosin-eosin stain technique; percentage of abnormal spermatozoa using 0.5% alcoholic eosin; sperm concentration ($\times 10^6$/ mL) estimated haemocytometrically and initial fructose concentration (mg / 100 mL semen) using the method described by Mann (1964). Semen was evaluated as described by Zemjanis (1962), El-Sherbiny (1987) and Madhuri *et al.* (2012).

**Fertility test:**

Remained male NZW rabbits (8 bucks from each group) at 6 months of age were used for artificial insemination. For testing the fertility of rabbit bucks, 80 hybrid nonparous females, were inseminated artificially using diluted semen from experimental males (20 females per each experimental group). Semen from each experimental group was pooled together and diluted with Tris-citric-glucose contained 20% egg yolk, as described by El-Sherbiny (2014). Females chosen for insemination were thought to be sexually receptive (had red color of vulva lips). In order to induce ovulation, females were injected intramuscularly with 0.25 ml receptal (GnRH analogue, 1.05 µg of busereline acetate; intervet, Cairo, Egypt). Then, each doe was inseminated artificially with 0.5 ml diluted semen (containing approximately $30 \times 10^6$ sperm cells) just after GnRH injection. Pregnancy was detected by trans-abdominal palpation 14 days post-insemination to determine conception rate. Litter size was determined for each doe directly after kindling.

**Statistical analysis:**

Data of body weight, weight gain, semen characteristics and biochemical parameters were analyzed using repeated measurements analysis. Anatomical data were analyzed using Two Way Analysis of Variance (ANOVA). Litter size, behavioral and histological data were analyzed using One Way ANOVA. Whereas, for pregnancy rate trait, Chi-square test for homogeneity of variance were performed. All statistical analysis for the different traits was realized using SAS program (SAS, 2011). Differences among experimental groups were tested by Duncan's Multiple Range test (Duncan, 1955).

Repeated measurements analysis was according to the following model:

$y_{ijk} = \mu + trt_i + an_k (trt)_i + time_j + (trt * time)_{ij} + e_{ijk}$; Where: µ is the overall mean, $y_{ijk}$ is the observation of the studied trait of $k^{th}$ animal of $i^{th}$trt in $j^{th}$ time, $trt_i$ is the effect of $i^{th}$trt (i = 1, 2, 3,4), $an_k (trt)_i$ is $k^{th}$ animal within $i^{th}$ treatment (the first error), $time_j$ is the effect of $j^{th}$ time (j = 1, 2, 3, 4, 5 and 6 for semen quality and j = 1, 2, 3 and 4 for blood plasma biochemical analysis), $(trt*time)_{ij}$ is the effect of the interaction between trt and time, $e_{ijk}$ is the individual error. Two way ANOVA was according to the following model:

$y_{ijk} = \mu + trt_i + time_j + (trt * time)_{ij} + e_{ijk}$; Where: µ is the overall mean, $y_{ijk}$ is the observation of the studied trait of $k^{th}$ animal of $i^{th}$ trt and $j^{th}$ time, $trt_i$ is the effect of $i^{th}$ trt (i = 1, 2, 3 and 4), $Time_{ej}$ is the effect of $j^{th}$ time (j = 1, 2, 3 and 4), $(trt*time)_{ij}$ is the effect of the interaction between trt and time, $e_{ijk}$ is the individual error. One way ANOVA was according to the following model:

$$Y_{ij} = \mu + trt_i + e_{ij}$$

Where: $\mu$ is the overall mean, $Y_{ij}$ is the observation of the studied trait of $j^{th}$ animal of $i^{th}$ treatment, $trt_i$ is the fixed effect of treatment (i = 1, 2 3 and 4), $e_{ij}$ is the individual error.

## RESULTS AND DISCUSSION

**Effects of royal jelly or/ and bee honey on :**

**Body weight and weight gain :**

Overall mean of absolute monthly body weight (kg) and body weight gain (gm) of control and royal jelly or/ and honey- treated male NZW rabbits from 2 until 6 months of age are given in Table 1 and illustrated in Figure 1. The results demonstrated that the effects of both age and treatment on both absolute body weight and body gain were significant ($P<0.05$). Absolute body weigh was significantly higher in all treatment groups than the control one , being the highest in the $4^{th}$ group treated with and bee honey. Royal jelly with or without honey-treated males had the higher values of absolute body weight than control group; while, male rabbits of group 4 (royal jelly and honey) showed the higher values than the other three groups. The present values of body weight are very close to values obtained for NZW rabbits by Amann and Lambaise (1967), Berger (1982a), El-sherbiny (1994) and Lebas *et al.* (1997). Increasing body weight of royal jelly treated males may be due to the effect of higher concentration of testosterone which has anabolic effects (Table 1 and Figure 4). Testosterone possesses anabolic effects (Hafez and Hafez, 2000).

The monthly body weight gain decreased with increasing age ( Fig.1 B ) , but the overall mean of gain for groups 3 and 4 was higher compared with the other two groups. Bee honey-treated males showed higher body gain than that of control. These findings are in agreement with those obtained by Afifi *et al.* (1989) in guinea pigs; El-Sherbiny (1994), Bonomi *et al.* (2001) and Elnagar *et al.* (2010) in rabbits. The results of the present study were in disagreement with those obtained in rats by Yang *et al.* (2012) who found that royal jelly did not impart a significant effect on the body weight of the male rats.

The onset of puberty is more closely related to body weight than to age (Hafez and Hafez, 2000).

**Table 1: Overall mean (±SE) of growth parameters, behavioral parameters, semen characteristics, biochemical blood parameters and fertility of male NZW rabbits treated with royal jelly or/ and bee honey.**

| | Control Group | 2nd group | 3rd group | 4th group |
|---|---|---|---|---|
| Absolute body weight (kg) | 2.56±0.01$^{D*}$ | 3.01±0.01$^{C*}$ | 3.12±0.01$^{B*}$ | 3.19±0.01$^{A*}$ |
| Body weight gain (gm/month) | 573.4±16.96$^{C*}$ | 729.0±14.26$^{B*}$ | 768.7±14.69$^{AB*}$ | 806.8±14.69$^{A*}$ |
| Mean testes weight (mg) | 753.75±13.06$^{D*}$ | 981.38±13.06$^{C*}$ | 1032.14±13.06$^{B*}$ | 1327.63±13.06$^{B*}$ |
| Mean testis index ($cm^3$) | 1.05±0.02$^{C*}$ | 1.29±0.02$^{B*}$ | 1.26±0.02$^{B*}$ | 1.53±0.02$^{A*}$ |
| Mean epididymis weight (mg) | 315.91±7.66$^{D*}$ | 414.42±7.66$^{C*}$ | 558.85±7.66$^{B*}$ | 558.85±7.66$^{A*}$ |
| Age of descending of testes (days) | 112.29±1.80$^{A*}$ | 90.73±1.74$^{B*}$ | 88.75±1.63$^{B*}$ | 86.30±1.63$^{B*}$ |
| Age at separation of penis from sheath (days) | 117.29±1.82$^{A*}$ | 97.33±1.76$^{B*}$ | 93.29±1.65$^{BC*}$ | 90.71±1.65$^{C*}$ |
| Age at fighting (days) | 136.79±1.33$^{A*}$ | 112.67±1.29$^{B*}$ | 112.47±1.21$^{B*}$ | 98.71±1.21$^{C*}$ |
| Age at 1st ejaculation (days) | 139.43±1.14$^{A*}$ | 125.67±1.10$^{B*}$ | 116.76±1.04$^{C*}$ | 109.06±1.04$^{D*}$ |
| Age at sperm appearance in ST (days) | 150.0±5.77$^{A*}$ | 135.0±5.77$^{AB*}$ | 120.0±5.77$^{B*}$ | 120.0±5.77$^{B*}$ |
| Age at sperm appearance in epididymal ducts (days) | 150.0±5.77$^{A*}$ | 135.0±5.77$^{AB*}$ | 120.0±5.77$^{B*}$ | 120.0±5.77$^{B*}$ |
| Reaction time (seconds) | 16.8 ± 0.65$^{B*}$ | 9.1 ± 0.53$^{A*}$ | 8.9 ± 0.53$^{A*}$ | 6.9± 0.53$^{C*}$ |
| pH | 6.8 ± 0.02$^{B*}$ | 6.9 ± 0.02$^{AB*}$ | 6.9 ± 0.02$^{A*}$ | 6.9± 0.02$^{AB*}$ |
| Ejaculate volume (mL) | 0.20 ± 0.02 $^{B*}$ | 0.47 ± 0.02 $^{A*}$ | 0.49 ± 0.02 $^{A*}$ | 0.47± 0.02 $^{A*}$ |
| Sperm progressive motility % | 70.0 ± 1.1 $^{B*}$ | 88.0 ± 0.9$^{A*}$ | 91.0 ± 0.9 $^{A*}$ | 93.0± 0.9 $^{A*}$ |
| Sperm concentration / ml (×$10^6$) | 226.51 ± 4.39$^{D*}$ | 273.44 ± 3.39$^{C*}$ | 340.73 ± 0.01$^{B*}$ | 372.41± 0.01$^{A*}$ |
| Dead sperms % | 18.85 ± 0.98$^{A*}$ | 8.11 ± 0.83$^{B*}$ | 6.26 ± 0.83$^{BC*}$ | 4.80± 0.83$^{C*}$ |
| Abnormal sperms % | 13.69 ± 0.24$^{A*}$ | 8.17 ± 0.19$^{B*}$ | 6.56 ± 0.19$^{C*}$ | 5.70± 0.19$^{D*}$ |
| Seminal plasma fructose (mg/100 mL) | 263.80 ± 14.54$^{B*}$ | 415.04 ± 14.54$^{AC*}$ | 376.95 ± 14.54$^{AB*}$ | 394.25± 14.54$^{A*}$ |
| Testosterone (ng / ml) | 1.00± 0.04$^{D*}$ | 1.20± 0.04$^{C*}$ | 1.32± 0.04$^{B*}$ | 1.43± 0.04 $^{A*}$ |
| Cholesterol (mg / dL) | 82.52± 1.35$^{D*}$ | 90.03± 1.35$^{C*}$ | 98.52± 1.35$^{B*}$ | 120.79± 1.35$^{A*}$ |
| AST (IU / L) | 36.76± 3.11$^{NS}$ | 42.32± 3.11$^{NS}$ | 40.07± 3.11$^{NS}$ | 38.08± 3.11$^{NS}$ |
| ALT (IU / L) | 12.60± 0.67$^{NS}$ | 12.25± 0.67$^{NS}$ | 12.30± 0.67$^{NS}$ | 13.07± 0.67$^{NS}$ |
| Conception rate % (20 doe) | 70 NS | 75 NS | 80NS | 90NS |
| Litter size at birth (No. Kids/ doe) | 4.0±0.20$^{C*}$ | 5.6±0.20$^{B*}$ | 6.7 ± 0.20$^{A*}$ | 7.0±0.19$^{A*}$ |

**Overall means within a row OvOverall means within a row with different superscript letters differ significantly (P< 0.05)**

**NS= non significant** ***P<0.05**

## Epididymal and testicular anatomy :

Mean weights of right and left testes and epididymis and testis index are given in Table 1 and Figure 2. Data of the present study showed that both age ( Fig. 2 A,B,C ) and treatment affected significantly (P<0.05) all the three

anatomical parameters studied. Royal jelly treated- males gave higher values than honey and control groups; while, males of the 2nd group had the higher values than the control group. El-Sherbiny (1994) showed that pre-pubertal male NZW rabbits treated with GnRH analogue reported higher testis and epididymis weight and higher testis index. Subsequent work has shown that in the rabbit, the growth of testes follows a sigmoid curve, increasing rapidly during puberty (Gaddum, 1964).

After birth, the testes develop less quickly than the rest of the body (Lebas *et al.*, 1997). From the age of five weeks they begin to grow very rapidly. The increased testis and epididymis weight for royal jelly or/ and bee honey-treated males may be due to the higher concentration of testosterone (Table 1 and Figure 4). Testosterone develops spermatogenesis and possesses anabolic effects (Hafez and Hafez, 2000).

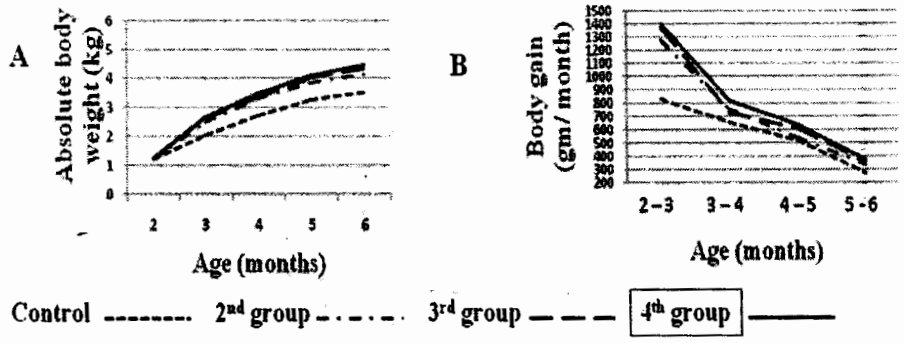


**Figure 1: Absolute body weight (A) and monthly body weight gain (B) of male NZW rabbits orally administered with royal jelly or/ and bee honey.**

**Behavioral phenomena:**

Effects of supplementing male NZW rabbits with royal jelly or/ and bee honey on ages at descending of testes into scrota, separation of penis from sheath, fighting and first ejaculated sperm are shown in Table 1. Differences among the experimental groups were significant ($P<0.05$). Royal jelly-treated males showed earlier age at descending testis into scrotum, separation of penis from sheath, fighting and first ejaculated sperm compared with honey and control males. The 4th group had earliest age at all traits studied. In the male rabbits, the testes remain in communication with the abdominal cavity, where they were at birth (Marshall, 1922 and Lebas *et al.*, 1997). Fighting age in the present study was later than those obtained by Skinner (1967), who observed that fighting began amongst male rabbits at 60 days of age and `bucking' (attempts to mount) about 10 days later.

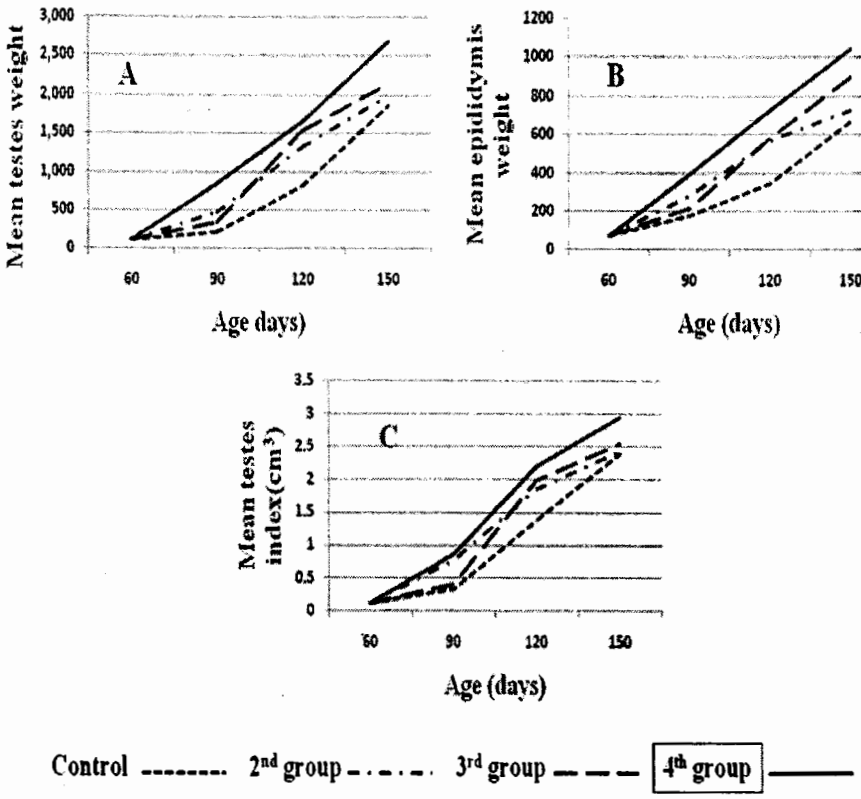


**Figure 2: Mean testis weight (mg), index ($cm^3$) and epididymis weight (mg) of male NZW rabbits orally administered with royal jelly or/ and bee honey.**

The rabbit is actually able to withdraw its testes when frightened or fighting with other males. The testicules descend at about two months (Lebas *et al.*, 1997). The short, back-slanting penis points forward when erect. The results of the present study are in agreement with those obtained by El-Sherbiny (1994) who found that male NZW rabbits injected with GnRH reflected earlier ages at the previous parameters.

Increasing body weight of males treated with royal jell or/ and bee honey (Table 1 and Figure 1) may influenced earlier age at sexual puberty. This may be in agreement with the findings of Alvariño (2000); Castellini (2008) and Rodríguez-De Lara *et al.* (2010), who reported that sexual behavior, sperm production and semen quality vary with body weight.

Testosterone develops sexual behavior (Hafez and Hafez, 2000). Lebas *et al.* (1997) reported that the first manifestations of sexual behavior appear at days 60 to 70 when the rabbit makes it first attempts at riding.

**Appearance of spermatozoa in seminiferous tubules and epididymal ducts:**

The averages of age at appearance of spermatozoa in the testis and epididymis are represented in Table 1. Calculated data showed that spermatozoa were present for the first time in seminiferous tubules and epididymal ducts of RJ treated- groups significantly earlier than honey and control groups. Basic structure of testis (seminiferous tubules cords and interstitial tissue) remains unchanged from gonadal sex differentiation at beginning of fetal life to onset of puberty (Hafez and Hafez, 2000). Spermatogenesis begins between days 40 and 50 (Lebas *et al.*, 1997). They reported also that testicular tubes become active at about 84 days. The first spermatozoa are present in the ejaculate at about 110 days. In the present study, spermatozoa appeared in testis and epididymis at 120 days for RJ treated males and 150 days in control bucks, this may be due to age of histological preparation at 90 and 120, 150 and 180 days (intervals of 30 days).

Testosterone is essential for spermatogenesis from spermatogonium to spermatid (West and Taylor, 1997). At puberty, gonocytes migrate to periphery of tubules; differentiate into spermatogonia; supporting cells produce Sertoli cells. These changes occure at the elevation of pre-pubertal gonadotropins (Hafez and Hafez, 2000).

**Semen characteristics of male NZW rabbits:**

Means of first and second ejaculation characteristics are given in Table 1 and Figure 3. Males treated with royal jelly or/ and honey had higher sexual activity reflected on lower reaction time (RT) compared with bucks in the control group ($P<0.05$). Lower reaction time for royal jelly or/ and honey-treated groups reflects the higher concentration of testosterone (Table 1 and Figure 4). Royal jelly or/ and honey treatments increased significantly ($P<0.05$) ejaculate volume, percentage of sperm progressive motility, sperm-cell concentration and seminal fructose concentration. While, 2$^{nd}$ group showed the highest fructose concentration among groups; this may be due to that honey contains high content of glucose and fructose as mentioned by Molan and Russell (1988) and Al-Waili (2004). On the other hand, control group showed significantly the highest percentages of dead and abnormal spermatozoa. The present results on the effect of royal jelly or/ and honey administration on libido and semen characteristics of male rabbits are in line with the findings of Elnagar (2010).

The values obtained in the present study are in agreements with those reported by Lebas *et al.* (1997) that in rabbit, volume of semen ejaculated is about 0.3 to 0.6 ml. and that sperm concentration is elevated at 150 days old to $500 \times 10^6$ spermatozoa / ml. Body weight of the mature male rabbit at semen collection had some influence on libido, semen and sperm characteristics (Skinner, 1967 and Hafez, 1970).

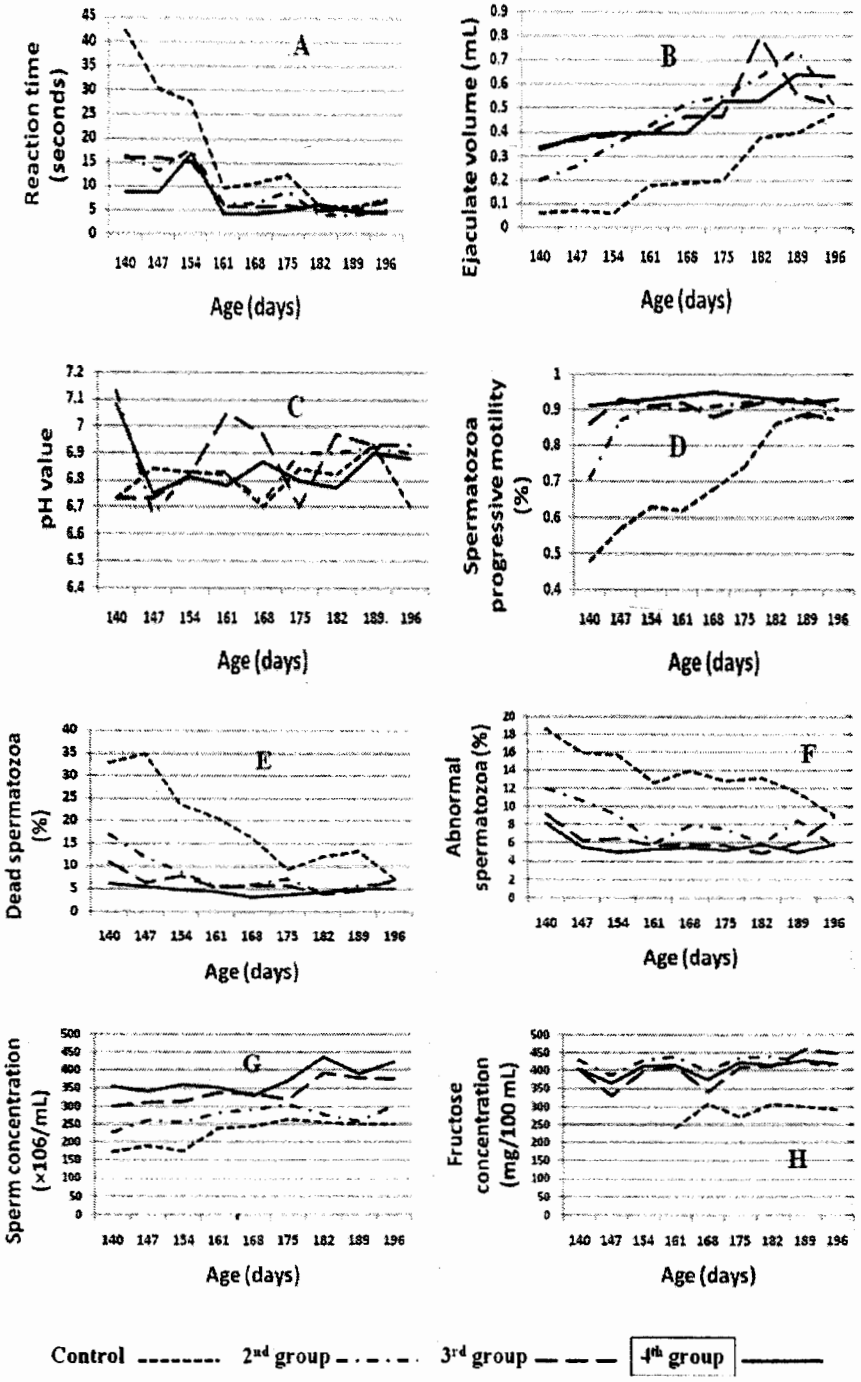


**Figure 3: Semen characterestics of of male NZW rabbits orally administered with royal jelly or/ and bee honey and control rabbits.**

The enhancement of semen quality in RJ or/ and H-treated male rabbits could be associated with higher concentration of testosterone recorded for these groups (Table 1 and figure 4), particularly libido of bucks, ejaculate volume and seminal plasma fructose which are testosterone dependent process (Nishiyama, 1955; Fujihara *et al.*, 1983; El-Sherbiny, 1994; Hafez and Hafez, 2000; Hashim *et al.*, 2013). Hafez and Hafez (2000) also stated that fructose synthesis by the accessory sex glands was dependent on the secretion of testosterone hormone by Leydig cells of testis. The enhancement observed in sperm motility is consistent with the findings of Karacal and Aral (2008) who reported higher sperm motility when male mice were treated with RJ. The significant increase in sperm-cell concentration can be explained by the findings of Kohguchi *et al.* (2004) who demonstrated that golden hamster treated with RJ showed more intensive spermatogenesis than the control group. Similar observations were recorded by Karacal and Aral (2008) who reported higher sperm concentration when male mice were treated with RJ. ElKelawy and Aboulnaga (1995) reported that ejaculate volume, sperm motility and sperm concentration were significantly increased when male rabbits treated with testosterone. In addition, RJ or/ and H treatments were capable of significantly reducing abnormal and dead sperm percentages. That comes in line with the findings of Karacal and Aral (2008) who reported lower abnormal sperm concentrations when male mice were treated with RJ, where the percentage of abnormal spermatozoa in the control group was higher than those of other groups.

Hassan (2009) concluded that royal jelly is a beneficial treatment of male adult rats, especially on sperm count and the percentage of live sperm percentage. On rabbits, Elnagar (2010) concluded that RJ administration to heat stressed male rabbits can counteract their "summer infertility".

Body weight in different mammalian species influences reproductive potential (Rodríguez-De Lara, 2015). Several studies in rabbits provided evidence that sperm production was influenced by body weight (Hafez, 1970; Alvariño, 2000; Castellini, 2008; Rodríguez-De Lara *et al.*, 2010). Increasing body weight for rabbits orally administered with royal jell or/ and bee honey (Table 1 and figure 1) may be produced better semen quality than control. Rodríguez-De Lara (2015) reported that body weight of the mature male rabbit had some influence on libido, semen and sperm characteristics; and in disagreement with that obtained by Brun *et al.* (2006) who found that males that weighed less from one of two lines divergently selected for body weight had greater semen volume, sperm motility and number of sperm per ejaculate that are suitable for AI than those of the line with greater weights.

In the present study, 2 successive ejaculates were collected separately and evaluated individually, then the mean of the two ejaculates were recorded. Lebas *et al.* (1997) reported that in two successive ejaculates, the first acts as a preparation for the second, which is less voluminous but more concentrated. Two ejaculates collected once a week (in a period of at least 15 min) give good semen production results (Bencheikh, 1995, Moce *et al.*, 2000).

**Plasma biochemical parameters and testosterone concentration:**

Data in Table 1 and Figure 4, showed that supplementation of royal jelly or/ and honey increased significantly (P<0.05) plasma testosterone and cholesterol concentrations compared with the control group.

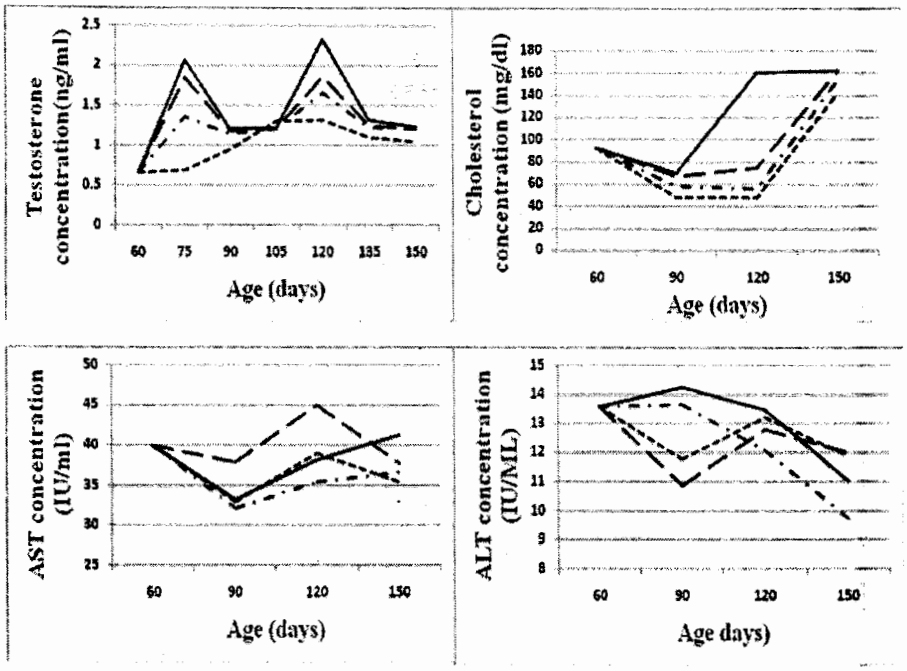


**Figure 4: Plasma concentrations of testosterone and cholesterol, and activity of AST and ALT in blood plasma of male NZW rabbits orally administered with royal jelly or/ and bee honey and control rabbits.**

On the other hand, blood plasma activities of AST and ALT differ non-significantly among the experimental groups.

The previous results are partly in agreements with the findings of Elnagar (2010).

Liver enzymes (ALT and AST) tend to rise suggesting some liver damage in mammals and birds (QingHua and Genlin, 2007; Faisal *et al.*, 2008). In the present study, RJ or/ and H treatments showed non-significant differences in both enzymes than control, revealed normal liver function. These effects are in agreement with the finding of Elnagar (2010).

**Male rabbits' fertility:**

Table 1 represents the effect of royal jelly or/ and bee honey on conception rate % (CR) and litter size at birth (LS) for rabbit does inseminated artificially with male rabbit semen of the four experimental groups. Chi-square test showed that, there were no significant differences

among experimental groups in conception rate. Values of CR were 70, 75, 80 and 90% for control, 2nd, 3rd, and 4th groups, respectively ( Table 1 ).

On the other hand, there were significant differences ($P<0.05$) among experimental groups on litter size (Table 1). Increased fertility of male rabbits treated with royal jelly or/ and bee honey may be due to the effect of high testosterone concentration that improved semen quality and resulted in high conception rate and litter size. The effect of testosterone on previous parameters was in line with El-Sherbiny (1994). Honey may improve semen quality and fertility of male rabbits as reported by Sayazana *et al.* (2011) who suggested that Gelam honey has the potential to increase the fertility of male rats by increasing sperm count and number of sperm with normal morphology.

## CONCLUSION

It could be concluded that oral administration of royal jelly or/ and bee honey to male NZW rabbits increased their body weight, testis weight, epididymis weight and testis index; resulted in earlier age at puberty and improved their reproductive performance as observed with higher testosterone concentration, libido, ejaculate volume, sperm progressive motility percentage, sperm-cell concentration, seminal plasma fructose concentrations , conception rate and litter size; also by decreasing abnormal and dead sperm percentages. This improvement was also mirrored on better liver functions as observed with normal activity of AST and ALT. The results of the present study showed that royal jelly or/ and bee honey at any level could be used beneficially to have earlier puberty age, improve semen quality and fertility of male rabbits.

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# عمر البلوغ الجنسى و الخصوبة لذكور الأرانب النيوزيلندى الأبيض بعد تجريعها بغذاء الملكات أو/ و عسل النحل

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صممت هذه التجربة لدراسة تأثير غذاء الملكات أو/ و عسل النحل على العمر عند البلوغ الجنسى، و جودة السائل المنوى، و خصوبة ذكور الأرانب النيوزيلندى الأبيض. أستخدم فى هذه الدراسة ٨٠ ذكر نيوزيلندى أبيض غير بالغ جنسيا و ٨٠ أنثى أرنب خليط لم تلد بعد. تم تقسيم ذكور الأرانب عشوائيا إلى ٤ مجموعات تجريبية (٢٠ ذكر لكل مجموعة)، حيث تم تجريع هذه الذكور بنصف ملليلتر محلول / كجم من وزن الجسم ٣ مرات أسبوعيا لمدة ٦ أسابيع، و احتوى هذا المحلول على: ١) ماء للمجموعة الضابطة، ٢) ٠,٢٥ مل عسل نحل + ٠,٢٥ مل ماء للمجموعة الثانية، ٣) ٢٠٠ مجم غذاء ملكات + ٠,٥ مل ماء للمجموعة الثالثة، ٤) ٢٠٠ مجم غذاء ملكات + ٠,٢٥ مل عسل نحل + ٠,٢٥ مل ماء و ذلك للمجموعة الرابعة.

أوضحت نتائج هذه الدراسة أن ذكور الأرانب النيوزيلندى الأبيض قبل البلوغ الجنسى المعاملة بغذاء الملكات أو/ و عسل النحل أظهرت تبكير معنوى (عند مستوى إحتمال 0.05) للعمر عند البلوغ الجنسى (عمر مبكر عند نزول الخصية فى كيس الصفن، و انفصال القضيب عن غلافه، و الشجار، و أول قذفة منوية، و ظهور الحيوانات المنوية فى الخصية و البربخ)، و زيادة معنوية (عند مستوى إحتمال 0.05) فى حجم القذفة المنوية، و النسبة المنوية للحيوانات المنوية المتحركة تقدميا، و تركيز الحيوانات المنوية، و تركيز الفركتوز فى بلازما السائل المنوى مقارنة بالمجموعة الضابطة. على الجانب الآخر، زاد معنويا (عند مستوى إحتمال 0.05) النسبة المئوية للحيوانات المنوية الميتة و الشاذة للمجموعة الضابطة عند مقارنتها بالمجموعات الثلاث الأخرى. تركيز التستستيرون و الكولستيرول فى بلازما الدم زاد معنويا (عند مستوى إحتمال 0.05) للمجموعات المعاملة بغذاء الملكات أو/ و عسل النحل مقارنة بالمجموعة الضابطة. فى حين أن نشاط AST و ALT فى بلازما الدم اختلف بدون معنوية بين المجموعات التجريبية. ذكور الأرانب المعاملة بغذاء الملكات أو/ و عسل النحل أظهرت خصوبة أفضل (معدل حمل و عدد خلفة أعلى) من المجموعة الضابطة.

**الخلاصة**: أظهرت نتائج هذه الدراسة أن تجريع ذكور الأرانب النيوزيلندى الأبيض بغذاء الملكات أو/ و عسل النحل بأي مستوي تم تطبيقه من الممكن استخدامه بأمان للحصول على عمر بلوغ جنسى مبكر و تحسين خصائص السائل المنوى و الخصوبة لهذه الذكور. هذا التحسين كان منعكسا أيضا على تحسن فى وظائف الكبد من التركيزات الطبيعية لكل من AST و ALT .